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Award Number: DAMD17-03-1-0211

TITLE: Role of Schwannomin and Paxillin in Cell Growth Control

PRINCIPAL INVESTIGATOR: Cristina Fernandez-Valle, Ph.D.

CONTRACTING ORGANIZATION: University of Central Florida
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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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12a. DISTRIBUTION / AVAILABILITY STATEMENT
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13. ABSTRACT (Maximum 200 Words)

The goal of this work is to determine the physiological effect of schwannomin-paxillin interaction on Schwann cell growth as it pertains to tumor formation in Neurofibromatosis type 2. We propose that the schwannomin-paxillin protein complex contributes to the growth arrest of Schwann cells by regulating the internalization dynamics of erbB2/erbB3 receptors following ligand binding. The aims are as follows:

Aim 1: To conduct binding and mapping studies to identify a putative direct binding site for erbB receptors on schwannomin.

Aim 2: To conduct functional studies to assess the role of schwannomin and paxillin in cytoskeleton reorganization, receptor association and internalization, and proliferation in response to cell stimulation with GGF.

Aim 3: To conduct mechanistic studies to identify components of erbB2/erbB3 signaling complexes associated with growth promotion and inhibition by schwannomin.

A No Cost Extension was granted due to a delay in acquiring the necessary personnel. We have hired an experienced full-time technician Elizabeth Baldwin and have trained a PhD student, Courtney Thaxton. Work began on the last 2 months of year one on Aim 2 of the study. We have found that effective phosphorylation of schwannomim on serine 518 requires interaction with paxillin and appears to be stimulated by cdc42. GFP-schwannomin serine 518 and paxillin binding mutants have been transfected into Schwann cells. Results indicate that schwannomin specifies the bipolar morphology of Schwann cells. S518A mutants are unipolar whereas the S518D mutants have excessive branching filopodia reminiscent of oligodendrocyte. Removal of paxillin binding domain 1 abolishes the phosphorylation effect on morphology.

14. SUBJECT TERMS
Neurofibromatosis type 2, Schwann cells, merlin, erbB2, paxillin

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Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction/Body

Funds were used in the last 2 months of year 1 due to a delay in personnel hiring. Personnel are now in place and the work will progress as originally proposed. A No Cost Extension has been granted (See Appendix 1).

We are reporting progress made during the last 2 months of year 1 (March & April) by Courtney Thaxton a PhD student and Elizabeth Baldwin, a full time technician and Jared Iacovelli, a senior graduate student.

The progress is listed below.

Key Research Accomplishments

Aim 2.

We have generated and sequenced point mutants at serine 518 to alanine and to serine to glutamic acid in full-length schwannomin and schwannomin lacking one or both paxillin binding domains. We are subcloning merlin mutant cDNAs into the GFP and HC-Red plasmids for protein localization.

We have transfected and imaged (Figure 1) GFP-schwannomin full length and paxillin binding domain mutants with Serine 518 point mutations into primary rat Schwann cells for confocal imaging to assess effects on cytoskeleton organization and morphology. Xpress-tagged schwannomin mutants have been transfected into CHO and RT4 cells with and without active and dominant negative forms of rac for biochemical experiments to assess the effects on serine 518 phosphorylation.

Reportable Outcomes

GFP-schwannomin full length and the S518A and S518 D mutants all localize to the cell periphery. The S518D mutant causes formation of prolific branching filopodia whereas the S518A mutant causes formation of unipolar Schwann cells with a lamellapodia at one pole and filopodia at the opposite pole. The morphologies observed with these mutants are abolished if the paxillin binding domain 1 is deleted from schwannomin, indicating that schwannomin must bind paxillin to target to the plasma membrane to exert the phosphorylation dependent effect on cell shape. Schwann cells transfected with dn rac have a morphology similar to full length schwannomin, suggesting that schwannomin inhibits rac. See Appendix 2.

Conclusions

Preliminary results indicate that phosphorylation of serine 518 is not required for localization of schwannomin to the plasma membrane and filopodia and lamellapodia. This indicates that schwannomin binding to paxillin does not require phosphorylation of serine 518 by Pak, a rac-activated serine/threonine kinase. It suggests that schwannomin firsts binds paxillin and targets to the membrane where local activation of p21 activated kinase by cdc42 or rac stimulates phosphorylation of serine 518 on schwannomin and the ensuing change in morphology.

AMENDMENT OF SOLICITATION/MODIFICATION OF CONTRACT

1. CONTRACT ID CODE	PAGE OF PAGES
S	1 | 2

2. AMENDMENT/MODIFICATION NO.	3. EFFECTIVE DATE	4. REQUISITION/PURCHASE REQ. NO.	5. PROJECT NO.(If applicable)
P00003	04-May-2004	W23RYX-3046-N610	

6. ISSUED BY	CODE	7. ADMINISTERED BY (If other than Item 6)	CODE
USA MED RESEARCH ACQ ACTIVITY 820 CHANDLER ST FORT DETRICK MD 21702-5014	W81XWH	USA MED RESEARCH ACQ ACTIVITY ATTN: KAREN STOTLER 301-619-6867 KAREN.STOTLER@AMEDD.ARMY.MIL FORT DETRICK MD 21702	W81XWH

8. NAME AND ADDRESS OF CONTRACTOR (No., Street, County, State and Zip Code)		
UNIVERSITY OF CENTRAL FLORIDA 12443 RESEARCH PARKWAY SUITE 207 ORLANDO FL 32826-3252		9A. AMENDMENT OF SOLICITATION NO.
		9B. DATED (SEE ITEM 11)
	X	10A. MOD. OF CONTRACT/ORDER NO. DAMD17-03-1-0211
CODE 9H673 FACILITY CODE	X	10B. DATED (SEE ITEM 13) 22-Apr-2003

11. THIS ITEM ONLY APPLIES TO AMENDMENTS OF SOLICITATIONS

☐ The above numbered solicitation is amended as set forth in Item 14. The hour and date specified for receipt of Offer ☐ is extended. ☐ is not extended.

Offer must acknowledge receipt of this amendment prior to the hour and date specified in the solicitation or as amended by one of the following methods: (a) By completing Items 8 and 15, and returning _____ copies of the amendment; (b) By acknowledging receipt of this amendment on each copy of the offer submitted; or (c) By separate letter or telegram which includes a reference to the solicitation and amendment numbers. FAILURE OF YOUR ACKNOWLEDGMENT TO BE RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER. If by virtue of this amendment you desire to change an offer already submitted, such change may be made by telegram or letter, provided each telegram or letter makes reference to the solicitation and this amendment, and is received prior to the opening hour and date specified.

12. ACCOUNTING AND APPROPRIATION DATA (If required)

13. THIS ITEM APPLIES ONLY TO MODIFICATIONS OF CONTRACTS/ORDERS. IT MODIFIES THE CONTRACT/ORDER NO. AS DESCRIBED IN ITEM 14.

☐ A. THIS CHANGE ORDER IS ISSUED PURSUANT TO: (Specify authority) THE CHANGES SET FORTH IN ITEM 14 ARE MADE IN THE CONTRACT ORDER NO. IN ITEM 10A.

☐ B. THE ABOVE NUMBERED CONTRACT/ORDER IS MODIFIED TO REFLECT THE ADMINISTRATIVE CHANGES (such as changes in paying office, appropriation date, etc.) SET FORTH IN ITEM 14, PURSUANT TO THE AUTHORITY OF FAR 43.103(B).

X C. THIS SUPPLEMENTAL AGREEMENT IS ENTERED INTO PURSUANT TO AUTHORITY OF:
USAMRAA General Terms and Conditions

☐ D. OTHER (Specify type of modification and authority)

E. IMPORTANT: Contractor [X] is not, ☐ is required to sign this document and return _____ copies to the issuing office.

14. DESCRIPTION OF AMENDMENT/MODIFICATION (Organized by UCF section headings, including solicitation/contract subject matter where feasible.)
No Cost Extension

Except as provided herein, all terms and conditions of the document referenced in Item 9A or 10A, as heretofore changed, remains unchanged and in full force and effect.

15A. NAME AND TITLE OF SIGNER (Type or print)		16A. NAME AND TITLE OF CONTRACTING OFFICER (Type or print) JOSEPH S. LITTLE / CONTRACTING OFFICER TEL: 301 619 2546 EMAIL: joseph.little@us.army.mil	
15B. CONTRACTOR/OFFEROR _____ (Signature of person authorized to sign)	15C. DATE SIGNED	16B. UNITED STATES OF AMERICA BY Joseph S. Little (Signature of Contracting Officer)	16C. DATE SIGNED 10-May-2004

EXCEPTION TO SF 30
APPROVED BY OIRM 11-84

30 105 04

STANDARD FORM 30 (Rev. 10-83)
Prescribed by GSA
FAR (48 CFR) 53.243

SECTION SF 30 BLOCK 14 CONTINUATION PAGE

SUMMARY OF CHANGES

SECTION 00010 - SOLICITATION CONTRACT FORM

CLIN 0001

The CLIN extended description has changed from TITLE: Role of Schwannomin and Paxillin in Cell Growth Control" PRINCIPAL INVESTIGATOR: Dr. Cristina Fernandez-Valle PERIOD OF PERFORMANCE: 28 April 2003 - 27 May 2007 (Research ends 27 Apr 2007) to TITLE: Role of Schwannomin and Paxillin in Cell Growth Control" PRINCIPAL INVESTIGATOR: Dr. Cristina Fernandez-Valle PERIOD OF PERFORMANCE: 28 April 2003 - 27 May 2008 (Research ends 27 Apr 2008).

DELIVERIES AND PERFORMANCE

The following Delivery Schedule item for CLIN 0001 has been changed from:

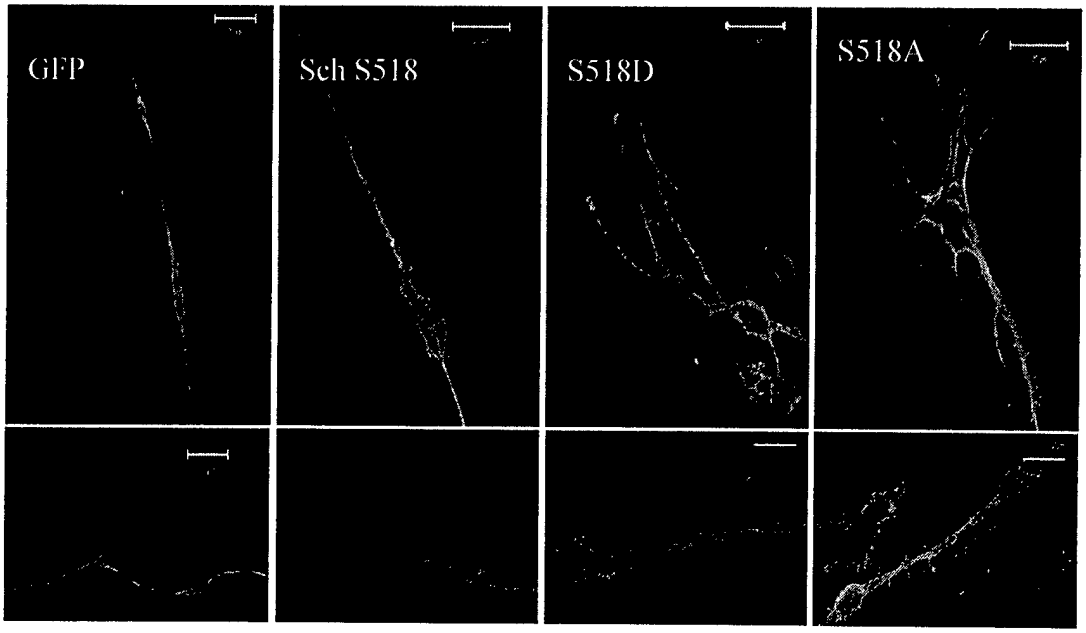
DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	UIC
POP 28-APR-2003 TO 27-MAY-2007	N/A	USA MED RESEARCH AND MATERIEL COM CDMRP 1053 PATCHEL STREET FORT DETRICK MD 21702-5012 FOB: Destination	W23RYX

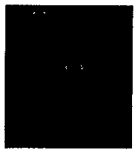
To:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	UIC
POP 28-APR-2003 TO 27-MAY-2008	N/A	USA MED RESEARCH AND MATERIEL COM CDMRP 1053 PATCHEL STREET FORT DETRICK MD 21702-5012 FOB: Destination	W23RYX

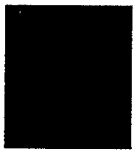
(End of Summary of Changes)

Transient Transfection of GFP-Sch S518 Mutants Into Schwann Cells.





GFP expressing SCs have bipolar filopodia.



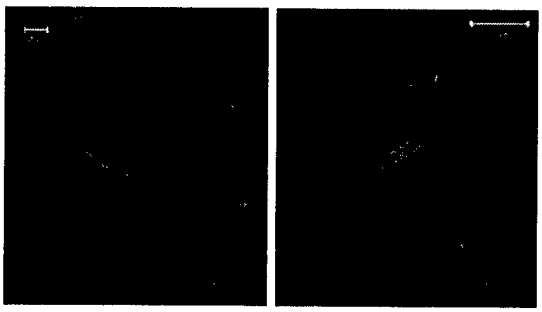
S518D expressing SCs have multiple branching filopodia.

S518 expressing SCs have long bipolar filopodia with many microvilli.

S518A expressing SCs are unipolar with a single filopodia and lamellapodia at opposing poles.

SCs were plated on laminin (25mg/mL) and were transiently transfected with 250ng of GFP alone and GFP Sch mutant cDNA plasmids for 4 hr. Cells were fixed, stained with Phalloidin (Alexa 633 Phalloidin, Molecular Probes) and were mounted 36 hours post-transfection. GFP Sch point mutations were constructed using a site directed mutagenesis (Stratagene Quick Change) on GFP Sch.

Transient Transfection of GFP-Sch DPBD1 With S518A or S518D



Deletion of PBD1 abolishes the phosphorylation dependent change in morphology. Transfected SCs have a bipolar morphology and resemble SCs transfected with GFP alone (see above).